



HAL
open science

TGF- β 2, catalase activity, H₂O₂ output and metastatic potential of diverse types of tumour

Malak Haidar, Mehdi Metheni, Frederic Batteux, Gordon Langsley

► To cite this version:

Malak Haidar, Mehdi Metheni, Frederic Batteux, Gordon Langsley. TGF- β 2, catalase activity, H₂O₂ output and metastatic potential of diverse types of tumour. Free Radical Biology and Medicine, 2019, 134, pp.282 - 287. 10.1016/j.freeradbiomed.2019.01.010 . hal-03486123

HAL Id: hal-03486123

<https://hal.science/hal-03486123v1>

Submitted on 20 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **TGF- β 2, catalase activity, H₂O₂ output and metastatic**
2 **potential of diverse types of tumour**

3 Malak Haidar^{1,2,3*}, Mehdi Metheni^{1,2*}, Frederic Batteux^{1,4} and Gordon
4 Langsley^{1,2#}

5 ¹Inserm U1016, Cnrs UMR8104, Cochin Institute, Paris, 75014 France.

6 ²Laboratoire de Biologie Cellulaire Comparative des Apicomplexes, Faculté de Médecine,
7 Université Paris Descartes - Sorbonne Paris Cité, France.

8 ³Present address: Pathogen Genomics Laboratory, Computational Bioscience Research
9 Center, King Abdullah University of Science and Technology (KAUST), Thuwal-23955-
10 6900, Kingdom of Saudi Arabia.

11 ⁴Laboratoire de Stress Oxydant, Prolifération Cellulaire et Inflammation, Faculté de
12 Médecine, Université Paris Descartes - Sorbonne Paris Cité, France.

13 *co-first authors, equally contributed

14 #Corresponding author: Gordon Langsley; Tel: +33 140516592; Fax: +33 140516570; Email:
15 gordon.langsley@inserm.fr

16 Keywords: *Theileria*; tropical theileriosis; TGF- β 2; H₂O₂; CREB; catalase; tumour
17 dissemination.

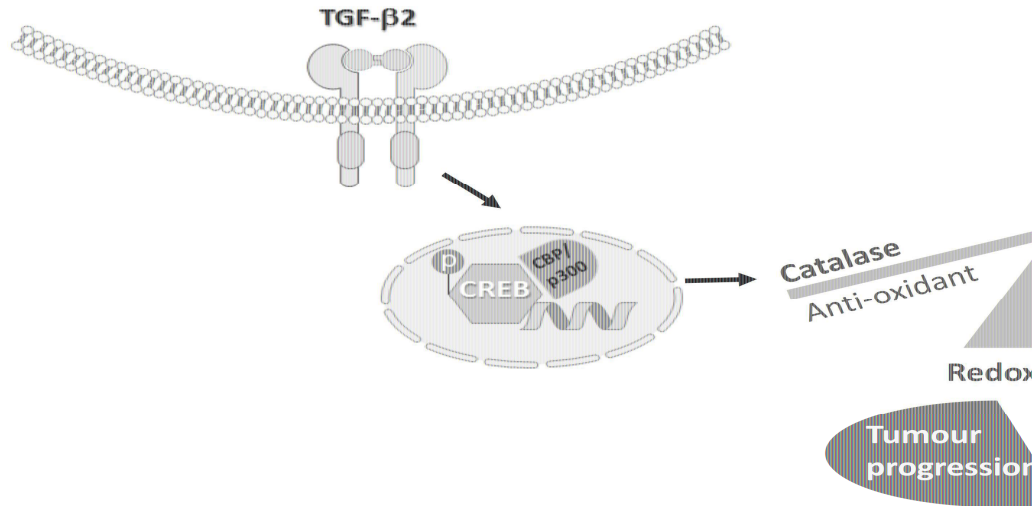
18 **Abstract:**

19 *Theileria annulata* is a protozoan parasite that infects and transforms bovine
20 macrophages causing a myeloid-leukaemia-like disease called tropical theileriosis.
21 TGF- β 2 is highly expressed in many cancer cells and is significantly increased in
22 *Theileria*-transformed macrophages, as are levels of Reactive Oxygen Species (ROS),
23 notably H₂O₂. Here, we describe the interplay between TGF- β 2 and ROS in cellular
24 transformation. We show that TGF- β 2 drives expression of *catalase* to reduce the
25 amount of H₂O₂ produced by *T. annulata*-transformed bovine macrophages, as well as
26 by human lung (A549) and colon cancer (HT-29) cell lines. *Theileria*-transformed
27 macrophages attenuated for dissemination express less catalase and produce more
28 H₂O₂, but regain both virulent migratory and matrigel traversal phenotypes when
29 stimulated either with TGF- β 2, or catalase to reduce H₂O₂ output. Increased H₂O₂
30 output therefore, underpins the aggressive dissemination phenotype of diverse tumour
31 cell types, but in contrast, too much H₂O₂ can dampen dissemination.

32 **Highlights**

- 33 • TGF- β 2 drives *catalase* transcription to reduce tumour H₂O₂ levels
- 34 • Blockade of TGF-R or CREB decreases catalase activity increasing H₂O₂
35 output
- 36 • Different tumours use TGF- β 2-driven *catalase* to control their H₂O₂ redox
37 balance
- 38 • TGF- β 2-mediated H₂O₂ output impacts on tumour metastatic potential

39 **Graphical abstract:**



40 **TGF-β2 drives catalase expression that reduces H₂O₂ levels and enhances**
41 **tumour dissemination**

42 **Introduction**

43 TGF-β (Transforming Growth Factor beta) is a pleiotropic cytokine involved in
44 diverse cellular processes such as proliferation, apoptosis and motility [1 , 2]. In
45 cancer TGF-β can act as an oncogenic factor that promotes tumour progression and
46 three isoforms have been defined of which TGF-β2 is highly expressed by many
47 tumours, especially those showing a high dissemination potential. *Theileria annulata*
48 parasitizes bovine macrophages and transforms them into disseminating tumours that
49 cause a myeloid-leukemia-like disease called tropical theileriosis. However, *T.*
50 *annulata*-transformed macrophage dissemination can be attenuated by multiple *in*
51 *vitro* passages and attenuated macrophages are used as live vaccines in countries
52 endemic for tropical theileriosis [3].

53 TGF- β 2 levels are high in *Theileria*-transformed disseminating macrophages and this
54 correlates with susceptibility to disease [4]. However, upon attenuation *in vitro* the
55 Ode vaccine line displays both reduced TGF- β 2 expression and dampened
56 dissemination, both of which are re-established by addition of exogenous TGF- β [4];
57 observations consistent with a pro-metastatic role for TGF- β 2 in the virulence of
58 *Theileria*-transformed macrophages. Furthermore, although virulent disseminating
59 macrophages display oxidative stress upon attenuation they actually produce more
60 H₂O₂ [5]. Heightened reactive oxygen species (ROS) have been reported to increase
61 TGF- β expression and stimulate release of TGF- β from latent complexes [6, 7] and
62 this likely contributes to TGF- β 2 production by virulent macrophages, but it does not
63 explain why attenuated macrophages that produce more H₂O₂ express less TGF- β 2 [4,
64 5]. TGF- β 2-signalling induces the transcription factor CREB in *Theileria*-transformed
65 macrophages and consequently, with loss of TGF- β 2 production CREB activity also
66 diminishes in attenuated macrophages [8]. The cyclic AMP response element-binding
67 protein (CREB) is a transcription factor of general importance in diverse cell types [9]
68 and CREB signalling is associated with cancer development and poor clinical
69 outcome in leukemogenesis [10], but it is not known if CREB is a player in ROS
70 regulation.

71 Elevated rates of ROS production have been described for human cancer cells, where
72 ROS underpins many aspects of tumour development and progression [11]. However,
73 too much ROS can be toxic for tumours [12] and to detoxify excessive ROS tumours
74 also express antioxidant proteins such as superoxide dismutases (SODs), catalase,
75 peroxiredoxins, the glutathione system that includes glutathione (GSH), glutathione
76 reductase and glutathione peroxidases (GPx) [11]. Here, we focus on catalase that

77 detoxifies hydrogen peroxide (H₂O₂) by turning it into water and oxygen. We report
78 for the first time that TGF-β2 induces CREB transactivation to promote *catalase*
79 transcription that leads to increased catalase activity and reduction in H₂O₂ levels. We
80 provide evidence that TGF-β2-driven catalase activity regulates the H₂O₂ redox
81 balance that impacts directly not only on the tropical theileriosis causing aggressive
82 dissemination phenotype of *Theileria*-transformed macrophages, but also on the
83 metastatic potential of human lung and colon cancer cell lines.

84 **MATERIALS AND METHODS**

85 **Cell culture:** virulent Ode macrophage line [13] corresponds to passage 62 and
86 attenuated macrophages correspond to passage 309. All macrophages were maintained
87 in RPMI medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-
88 glutamine, 100U penicillin, 0.1 mg/ml streptomycin, and HEPES. The cell lines S2,
89 S3 and H7–H8 have been described previously [14]. Cells were maintained in the
90 above culture medium with 50 μM of β-mercaptoethanol. The human colon
91 adenocarcinoma cell line HT-29 was maintained in McCoy's medium supplemented
92 with 10% FBS. A549 human lung adenocarcinoma cells (ATCC, CCL-185) were
93 cultured in DMEM/RPMI (1:1) with 10% fetal bovine serum. All the cells were
94 incubated at 37°C with 5% CO₂.

95 **Total RNA extraction and reverse transcription:** Total RNA of *Theileria*-infected
96 macrophages was isolated using the RNeasy mini kit (Qiagen) according to the
97 manufacturer's instructions. The quality and quantity of RNA was measured by
98 Nanodrop spectrophotometer. For reverse transcription, 1μg isolated RNA was
99 diluted in water to a final volume of 12μL, warmed at 65°C for 10 min, then
100 incubated on ice for 2 min. Afterwards, 8μl of reaction solution (0.5μL random
101 hexamer, 4μL 5x RT buffer, 1.5μL 10mM dNTP, 1μL 200U/μLRT-MMLV

102 (Promega) and 1 μ L 40U/ μ LRNase inhibitor (Promega) was added to get a final
103 reaction volume of 20 μ L and incubated at 37°C for 2 h. The resultant cDNA was
104 stored at -20°C.

105 **Quantitative polymerase chain reaction (qPCR):** mRNA expression levels were
106 estimated by qPCR on Light Cycler 480 (Roche) using SYBR Green detection
107 (Thermo). *GAPDH* was used as internal control to normalize for mRNA levels. The
108 detection of a single product was verified by dissociation curve analysis and relative
109 quantities of mRNA calculated using the method described [15].

110 **Pharmaceutical inhibition and activation:** TGF- β signalling was inhibited using the
111 TGF-Receptor I/ALK5 inhibitor SB431542, 10 μ M (Sigma #S4317) and activated by
112 adding 5ng/ml of recombinant bovine TGF- β 2 (NIBSC, Potters Bar. UK). Cells were
113 treated for 24 h at 37°C. Catalase activity was ablated with a selective inhibitor
114 Aminotriazole (AT) (Sigma, A8056), and restored by adding bovine catalase (Sigma,
115 C4963-2MG). Cells were treated overnight at a concentration of 1200 μ M with AT
116 and 80U/ml of bovine catalase. For inhibiting CREB transcription, a cell-permeable
117 naphthamide compound that effectively blocks the interaction between the KIX
118 domain of CBP and the KID domain of CREB was used (Calbiochem, CAS 92-78-4)
119 at a concentration of 25 μ M for 1 h at 37°C with 5% CO₂.

120 **Western Blotting:** Cells were harvested and extracted by lysis buffer (20mM Hepes,
121 Nonidet P40 (NP40) 1%, 0.1% SDS, 150mM NaCl, 2mM EDTA, phosphatase
122 inhibitor cocktail tablet (PhosSTOP, Roche) and protease inhibitor cocktail tablet
123 (Complete mini EDTA free, Roche). Protein concentration was determined by the
124 Bradford protein assay. Cell lysates were subjected to Western blot analysis using
125 conventional SDS/PAGE and protein transfer to nitrocellulose filters (Protran,
126 Whatman). The membrane was blocked by 5% non-fat milk-TBST (for anti-catalase),

127 or 3% non-fat milk-PBST (for anti-actin antibody) for 90 min at room temperature
128 (RT).

129 Antibodies used in immunoblotting were as follows: rabbit polyclonal antibody anti-
130 catalase (Cell Signaling, # 14097S) and goat polyclonal antibody anti-actin (I-19,
131 Santa Cruz Biotechnology, # sc-1616). Membranes were incubated with peroxidase-
132 conjugated secondary antibody (rabbit anti-IgG (#sc-2313) and goat anti-IgG (sc-
133 2020); Santa Cruz biotechnology). The dilutions used were 1/500 for the catalase
134 antibody, 1/2000 for anti-actin and 1/5000 for both secondary antibodies. After
135 washing, proteins were visualized with ECL western blotting detection reagents
136 (Thermo Scientific) on X-ray films. The level of β -actin was used as a loading control
137 throughout.

138 **Dynamic monitoring of cell migration with the xCELLigence system:** Cell
139 migration assay was assessed using the xCELLigence system (Roche). Medium was
140 added to the bottom chamber of the CIM-Plate 16. The CIM-Plate 16 was assembled
141 by placing the top chamber coated with Matrigel (BD) onto the bottom chamber and
142 snapping the two together. Serum-free medium was placed in the top chamber to
143 hydrate and the membrane was pre-incubated for 1 h in the CO₂ incubator at 37°C.
144 Once the CIM-Plate 16 has equilibrated, it is placed in the RTCA DP station and the
145 background cell-index values are measured. The CIM-Plate 16 is then removed from
146 the RTCA DP station and the cells passaged 24h in serum free medium were added to
147 the top chamber. The CIM-Plate 16 is placed in the RTCA DP station and migration is
148 monitored for several hours.

149 **Matrigel chambers assay:** The invasive capacity of transformed cells was assessed
150 *in vitro* using Matrigel migration chambers, as described [16]. Culture coat 96-well
151 medium BME cell invasion assay was obtained from Cultrex instructions (3482-096-

152 K). After 24 h of incubation at 37°C, each well of the top chamber was washed once
153 in buffer. The top chamber was placed back on the receiver plate. 100µL of cell
154 dissociation solution/Calcein AM were added to the bottom chamber of each well,
155 incubated at 37°C for 1 h to fluorescently label cells and dissociate them from the
156 membrane before reading at 485 nm excitation, 520 nm emission using the same
157 parameters as the standard curve.

158 **Intracellular levels of hydrogen peroxide (H₂O₂):** 1×10⁵ cells were seeded in 96
159 well plates and incubated 18h in complete medium. Cells were washed in PBS and
160 incubated with 100µL per well of 5µM H₂-DCFDA diluted in PBS (Molecular
161 Probes). H₂O₂ levels were assayed by spectrofluorimetry on a fusion
162 spectrofluorimeter (PackardBell). Fluorescence intensity was recorded every hour
163 over a period of 5 h. Excitation and emission wavelengths used for H₂O₂ were 485
164 and 530nm. The number of cells was evaluated by the crystal violet assay. Cells were
165 stained in 0.05% crystal violet and 2% ethanol in PBS for 30 min at room
166 temperature. After four washes in PBS, the stain was dissolved in methanol and
167 measured at 550nm on Fusion. The level of H₂O₂ was calculated in each sample as
168 follows: reactive oxygen species rate (arbitrary unitsmin⁻¹10⁵ cells⁻¹) = [fluorescence
169 intensity (arbitrary units) at T300 minutes – fluorescence intensity (arbitrary units) at
170 T0] per 60min per number of cells as measured by the crystal violet assay.

171 **Catalase activity assay:** A dry pellet of 1×10⁵ cells was lysed in 50µL PBS, 1%
172 NP40. 50µL of lysate, 50µL of anti-peroxydase antibody (1/2000, Sigma) and 50µL
173 H₂O₂ (1/4000, Sigma) were added to a 96-well plate and incubated for 10 min at 37°C
174 in 5% CO₂. 50µL of OPD (SIGMAFAST™, #P9187) was then added and the
175 absorbance immediately read at 405nm. Catalase activity assay was assayed on

176 Fusion. Catalase measurement was reported to the amount of protein in each sample
177 (bovine serum albumin microbiuret assay, Pierce, Bezons, France).

178 **SOD activity:** Superoxide dismutase (SOD) activities of cells were evaluated by the
179 nitroblue tetrazolium reduction technique according to Beauchamp and Fridovich
180 [17]. SOD measurements were reported to the amount of protein in each sample
181 (Bradford method was used).

182 **Statistical Analysis:** All data represent average values obtained from 3 independent
183 experiments. Results were statistically analyzed in GraphPad Prism (version 7.0,
184 GraphPad Software Inc., San Diego, CA) and presented as mean+/-SEM. Statistically
185 significant differences when means of only two groups were assessed by t-test, but
186 when means of more than two groups are compared by one way repeated-measured
187 ANOVA. Values were considered to be significantly different when p values were <
188 0.05.

189 **Results**

190 ***T. annulata*-transformed macrophages attenuated for dissemination display**
191 **significantly less catalase activity compared to virulent hyper-invasive**
192 **macrophages.**

193 We previously showed that attenuation of *Theileria*-transformed macrophage
194 virulence correlates with an increase of H₂O₂ output [5]. This appeared counter-
195 intuitive, as one imagined that a decrease in infected macrophage virulence would be
196 accompanied by a reduction in their oxidative stress status. However, accumulation of
197 H₂O₂ can occur either due to an increase in superoxide dismutase (SOD) activity that
198 produces H₂O₂, or reduced detoxification of H₂O₂ leading to its accumulation. In
199 order to discriminate between these two possibilities SOD and catalase activities were

200 measured in virulent (V) and attenuated (A) *Theileria*-transformed macrophages
201 (Figure 1). No significant change in SOD activity was detected between virulent and
202 attenuated macrophages (Fig. 1A), whereas catalase activity was significant
203 diminished in attenuated macrophages (Fig. 1B). Moreover, decreased catalase
204 protein levels underpinned the reduced catalase activity of attenuated macrophages
205 (Fig. 1C). Catalase activity was also measured in *Theileria*-transformed macrophages
206 isolated from disease-resistant Sahiwal cattle and found to be lower (Fig.S1A). Thus,
207 attenuated macrophages isolated from disease-susceptible animals resemble
208 transformed macrophages isolated from disease-resistant animals with respect to
209 catalase activity.

210 **TGF β -2 stimulates *catalase* transcription leading to an increase in protein levels**
211 **and catalase activity.**

212 We examined if the decrease in TGF- β 2 levels underpinned loss of catalase
213 transcription and activity. When attenuated macrophages were stimulated with
214 recombinant TGF- β 2 *catalase* transcription is increased, but not that of *glutathione*
215 *peroxidase*, coding for another antioxidant enzyme (Figure 2A). Stimulation of
216 attenuated macrophages with recombinant TGF- β 2 increased catalase activity (Fig.
217 2B) and decreased H₂O₂ output (Fig. 2C). Taken together, TGF- β 2-signalling clearly
218 activates *catalase* transcription leading to increased amounts of catalase and greater
219 catabolic activity towards H₂O₂.

220 **TGF- β 2-signalling activates *catalase* transcription via CREB.**

221 CREB is a transcription factor that binds DNA on CRE sites (cAMP Response
222 Element) to regulate transcription of target genes, and in *Theileria*-transformed

223 macrophages TGF- β 2-signalling activates *CREB* transcription [8]. Consistently,
224 bioinformatic analyses detected CRE sites in the promoter region of the *catalase* gene
225 (data not shown). Inhibition of CREB-mediated transcription with a specific CREB-
226 CBP interaction inhibitor decreased *catalase* transcription and catalase activity to
227 levels characteristic of attenuated macrophages (Figure 3A & B). Consequently,
228 virulent macrophages produce H₂O₂ equivalent to attenuated levels and conversely,
229 decreased H₂O₂ output occurred upon activation of CREB-mediated transcription
230 following stimulation of attenuated macrophages with membrane-permeable db-
231 cAMP (Fig. 3C). Importantly, pre-treatment of attenuated macrophages with the
232 CREB inhibitor prevented the drop in H₂O₂ levels provoked by addition of db-cAMP
233 (Fig. 3C). Thus, TGF- β 2-signalling via CREB activates *catalase* transcription leading
234 to increased catalase activity and reduced H₂O₂ output.

235 **Virulence and attenuation of *Theileria*-transformed macrophages depends on**
236 **their redox balance.**

237 *T. annulata* transforms host macrophages into tumour-like cells that have heightened
238 motility and invasiveness two traits that are typical of metastatic/disseminating cancer
239 cells. Figure 4 shows that detoxifying H₂O₂ by adding catalase, or TGF- β 2 to
240 attenuated macrophages induces a regain in cell migration by *Theileria*-transformed
241 macrophages. Boiling catalase to inactive the enzyme ablated its ability to reduce
242 H₂O₂ levels that stimulate migration. By contrast, increasing H₂O₂ output by virulent
243 macrophages via SB431542 blockade of TGF-R attenuated their migration (Fig. 4).
244 Similarly, TGF-R blockade with SB431542 or inhibition of catalase activity with
245 AminoTriazole (AT) reduced matrigel traversal of virulent macrophages to levels
246 typical of attenuated macrophages (Fig.4B). Therefore, modifying transformed

247 macrophage redox balance via TGF- β 2 stimulation, or manipulating catalase activity
248 changes a virulence trait (heightened migration) of *Theileria*-transformed
249 macrophages.

250 **TGF β -2 stimulation also increases catalase activity and metastatic potential of**
251 **human lung and colon cancer cell lines.**

252 In order to extend our observations on *Theileria*-transformed macrophages to other
253 cancer cell types, we treated HT-29 (human colorectal adenocarcinoma) and A549
254 (adenocarcinomic human alveolar basal epithelial) cell lines with TGF-R and/or
255 CREB-CBP interaction inhibitors. Both HT-29 and A549 H₂O₂ levels increase
256 following inhibitor treatment due to a corresponding drop in catalase activity (Figure
257 5 A& B), similar to *Theileria*-transformed macrophages (Figures 2, 3 & FigS1).
258 Moreover, inhibition of TGF-R and/or CREB signalling decreases matrigel traversal
259 of A549 cells (Fig. 5C). The ensembles lead to the conclusion that TGF- β 2 regulation
260 of catalase activity via CREB-mediated transcription and their impact on H₂O₂-type
261 oxidative stress is common to different cancer cell types of human and bovine origin.

262 **Discussion**

263 In this study, we have demonstrated that TGF- β 2 induces CREB to drive *catalase*
264 transcription, leading to more catalase enzyme and hence activity to detoxify H₂O₂.
265 *Theileria*-transformed macrophages with attenuated dissemination potential are
266 characterized by decreased TGF- β 2 production, and consequently, reduced catalase
267 activity and increased H₂O₂ output. Stimulating attenuated macrophages with
268 exogenous TGF- β 2 increased catalase activity and decreased H₂O₂ output leading to a
269 regain in their capacity to migrate and traverse matrigel. In contrast, blockade of

270 either TGF-R or CREB binding to CBP decreased catalase activity and increased
271 H₂O₂ output led to a reduced migratory and matrigel traversal capacity of virulent
272 macrophages. These observations are not restricted to *Theileria*-transformed bovine
273 macrophages, but were shared by human A549 and HT-29 cancer cell lines, where
274 inhibition of TGF-β2-signalling and/or CREB-mediated transcription again decreased
275 catalase activity, increased the H₂O₂ output and reduced their capacity to traverse
276 matrigel. Thus, our demonstration that catalase activity and hence H₂O₂ levels are
277 regulated by TGF-β2-signaling can be generalized to different types of tumours.

278 Our study is consistent with a pro-metastatic role for TGF-β2, since adding back
279 recombinant TGF-β2 to attenuated macrophages resulted in a regain in their migratory
280 and dissemination potentials. Clearly, one way that TGF-β2 promotes virulent
281 dissemination is by inducing CREB transactivation to activate catalase and detoxify
282 excess H₂O₂. Tumours produce large amounts of ROS that cause damage to DNA,
283 proteins and lipids and we propose that infected macrophages attenuated for
284 dissemination have countered the tumorigenic effect of *Theileria* by producing higher
285 levels of H₂O₂. In virulent macrophages with high TGF-β2 levels CREB induction
286 activates catalase that dampens H₂O₂ output and similarly TGF-β2-mediated changes
287 H₂O₂ output and catalase activity impact on the metastatic potential of human A549
288 and HT-29 cancer cell lines. This argues that many tumours of different origins
289 exploit TGF-β2-driven *catalase* expression to control their H₂O₂ redox balance.

290 **Conclusion:**

291 We've shown that TGF-β2 regulates the oxidative stress status of *Theileria*-
292 transformed macrophages and two different types of human tumour. TGF-β2 drives
293 transcription of *catalase* through CREB signalling, which results in reduced H₂O₂

294 output promoting a regain of matrigel traversal phenotypes of both human and bovine
295 tumours.

296 **Acknowledgements:**

297 We thank Arnab Pain for fruitful discussion and input when writing the manuscript.
298 MH was supported by a PhD CNR fellowship from the Lebanese government. GL
299 acknowledges core support from INSERM and the CNRS.

300 **Funding:** This work was supported by the ANR grant (11 BSV3 01602), and Labex
301 ParaFrap (ANR-11-LABX-0024).

302 **Competing interests:**

303 The authors declare they have no competing financial interests in relation to the work
304 described.

305 **References:**

- 306 1. Chen W, Ten Dijke P. Immunoregulation by members of the TGFbeta
307 superfamily. *Nat Rev Immunol.* 2016;16(12):723-40. doi: 10.1038/nri.2016.112.
308 PubMed PMID: 27885276.
- 309 2. Cantelli G, Crosas-Molist E, Georgouli M, Sanz-Moreno V. TGFbeta-induced
310 transcription in cancer. *Semin Cancer Biol.* 2016. doi:
311 10.1016/j.semcancer.2016.08.009. PubMed PMID: 27586372.
- 312 3. Somerville RP, Adamson RE, Brown CG, Hall FR. Metastasis of *Theileria*
313 *annulata* macroschizont-infected cells in scid mice is mediated by matrix
314 metalloproteinases. *Parasitology.* 1998;116 (Pt 3):223-8. PubMed PMID: 9550215.
- 315 4. Chaussepied M, Janski N, Baumgartner M, Lizundia R, Jensen K, Weir W, et
316 al. TGF-b2 induction regulates invasiveness of *Theileria*-transformed leukocytes and
317 disease susceptibility. *PLoS Pathog.* 2010;6(11):e1001197. PubMed PMID:
318 21124992.
- 319 5. Metheni M, Echebli N, Chaussepied M, Ransy C, Chereau C, Jensen K, et al.
320 The level of H(2)O(2) type oxidative stress regulates virulence of *Theileria*-
321 transformed leukocytes. *Cell Microbiol.* 2014;16(2):269-79. PubMed PMID:
322 24112286.
- 323 6. Annes JP, Munger JS, Rifkin DB. Making sense of latent TGFbeta activation.
324 *Journal of cell science.* 2003;116(Pt 2):217-24. PubMed PMID: 12482908.
- 325 7. Glasauer A, Chandel NS. Targeting antioxidants for cancer therapy.
326 *Biochemical pharmacology.* 2014;92(1):90-101. doi: 10.1016/j.bcp.2014.07.017.
327 PubMed PMID: 25078786.

- 328 8. Haidar M, Echebli N, Ding Y, Kamau E, Langsley G. Transforming growth
329 factor beta2 promotes transcription of COX2 and EP4, leading to a prostaglandin E2-
330 driven autostimulatory loop that enhances virulence of *Theileria annulata*-transformed
331 macrophages. *Infection and immunity*. 2015;83(5):1869-80. doi: 10.1128/IAI.02975-
332 14. PubMed PMID: 25690101; PubMed Central PMCID: PMC4399038.
- 333 9. Mayford M, Abel T, Kandel ER. Transgenic approaches to cognition. *Curr*
334 *Opin Neurobiol*. 1995;5(2):141-8. PubMed PMID: 7620300.
- 335 10. Shankar DB, Sakamoto KM. The role of cyclic-AMP binding protein (CREB)
336 in leukemia cell proliferation and acute leukemias. *Leukemia & lymphoma*.
337 2004;45(2):265-70. PubMed PMID: 15101710.
- 338 11. Liou GY, Storz P. Reactive oxygen species in cancer. *Free radical research*.
339 2010;44(5):479-96. doi: 10.3109/10715761003667554. PubMed PMID: 20370557;
340 PubMed Central PMCID: PMC3880197.
- 341 12. Laurent A, Nicco C, Chereau C, Goulvestre C, Alexandre J, Alves A, et al.
342 Controlling tumor growth by modulating endogenous production of reactive oxygen
343 species. *Cancer research*. 2005;65(3):948-56. PubMed PMID: 15705895.
- 344 13. Singh DK. Methods currently used for the control of *Theileria annulata*: their
345 validity and proposals for future control strategies. *Parassitologia*. 1990;32(1):33-40.
346 PubMed PMID: 2126620.
- 347 14. McGuire K, Manuja A, Russell GC, Springbett A, Craigmile SC, Nichani AK,
348 et al. Quantitative analysis of pro-inflammatory cytokine mRNA expression in
349 *Theileria annulata*-infected cell lines derived from resistant and susceptible cattle.
350 *Veterinary immunology and immunopathology*. 2004;99(1-2):87-98. doi:
351 10.1016/j.vetimm.2004.01.003. PubMed PMID: 15113657.
- 352 15. Pfaffl MW. A new mathematical model for relative quantification in real-time
353 RT-PCR. *Nucleic acids research*. 2001;29(9):e45. PubMed PMID: 11328886;
354 PubMed Central PMCID: PMC55695.
- 355 16. Lizundia R, Chaussepied M, Huerre M, Werling D, Di Santo JP, Langsley G.
356 c-Jun NH2-terminal kinase/c-Jun signaling promotes survival and metastasis of B
357 lymphocytes transformed by *Theileria*. *Cancer research*. 2006;66(12):6105-10. doi:
358 10.1158/0008-5472.CAN-05-3861. PubMed PMID: 16778183.
- 359 17. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an
360 assay applicable to acrylamide gels. *Analytical biochemistry*. 1971;44(1):276-87.
361 PubMed PMID: 4943714.

362 **Figure legends:**

363 **Figure 1: Catalase activity is decreased in attenuated *Theileria*-transformed**
364 **macrophages. A.** SOD activity doesn't change between virulent and attenuated
365 macrophages. **B.** Catalase activity is diminished in attenuated compared to virulent
366 macrophages. **C top,** Catalase expression is high in virulent macrophages compared
367 to attenuated macrophages. **C bottom,** Actin expression is unchanged between
368 virulent and attenuated macrophages. ROS measurements in A and B were done
369 independently (n = 3) and in triplicate. ***p<0.001 compared to attenuated
370 macrophages.

371 **Figure 2: TGF- β 2 activates *catalase* transcription in *Theileria*-transformed**
372 **macrophages. A.** The transcription of *catalase* and *GPx* is decreased in attenuated
373 compared to virulent macrophages. Adding recombinant TGF- β 2 restores
374 transcription of *catalase* in attenuated macrophages to virulence levels. No effect was
375 observed in the transcription level of *GPx*. **B.** Left panel, Catalase activity is down
376 regulated in attenuated macrophages and rescued by exogenous TGF- β 2 stimulation.
377 **C.** Right panel, H₂O₂ levels are increased in attenuated compared to virulent
378 macrophages and is dampened by addition of exogenous TGF- β 2. All experiments
379 were done independently (n = 3) and in triplicate. *** p<0.001 compared to virulent
380 macrophages and ### p<0.001 compared to attenuated macrophages.

381 **Figure 3: CREB drives *catalase* transcription. A.** *Catalase* transcription is higher in
382 virulent (V) than attenuated (A) macrophages. Catalase transcription is diminished to
383 attenuated levels when CREB-mediated transcription in virulent macrophages is
384 ablated with the specific CREB-CBP interaction inhibitor (25 μ M 1 h at 37°C). **B.**
385 Catalase activity is higher in virulent (V) compared to attenuated (A) macrophages,

386 and CREB-CBP-mediated inhibition of CREB in virulent macrophages reduces
387 catalase activity to attenuated levels. **C.** H₂O₂ levels are higher in attenuated (A)
388 compared to virulent (V) macrophages. CREB-CBP-mediated inhibition of CREB-
389 driven transcription in virulent macrophages increases the level of H₂O₂, while adding
390 db-cAMP to attenuated macrophages decreases H₂O₂ output. Treatment with CREB-
391 CBP interaction inhibitor abolishes *catalase* expression and ablates the increase in
392 H₂O₂ induced by db-cAMP stimulation. All experiments were done independently (n
393 = 3) and in triplicate. *** p<0.001 compared to virulent macrophages, ## p<0.005
394 compared to attenuated macrophages and ### p<0.001 compared to attenuated
395 macrophages.

396 **Figure 4: TGF-β2 levels regulate both oxidative stress and metastatic potential of**
397 ***Theileria*-transformed macrophages. A.** Cell migration index of virulent (V)
398 macrophages is greater than that of attenuated (A) macrophages, but is reduced upon
399 TGF-R blockade with SB431542. TGF-β2 stimulation of attenuated macrophages
400 restores their cell migration index, as does addition of active catalase. Boiled catalase
401 fails to restore attenuated macrophages migration index. **B.** Matrigel traversal is
402 higher for virulent (V) than attenuated (A) macrophages. Blocking TGF-R-signalling
403 with SB431542 diminishes traversal of V macrophages, as does AT-induced
404 inhibition of catalase activity. All experiments were done independently (n = 3) and in
405 triplicate. *** p<0.001 compared to virulent macrophages and ### p<0.001 compared
406 to attenuated macrophages.

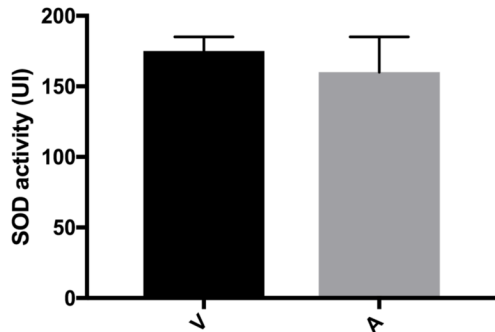
407 **Figure 5: Observations on *Theileria*-transformed macrophages can be extended**
408 **to human A549 and HT-29 cancer cell lines. A.** H₂O₂ levels are lower in HT-29 and
409 A549 compared to HT-29 and A549 treated with TGF-R inhibitor SB431542. **B.**
410 Catalase activity is higher in HT-29 and A549 compared to HT-29 and A549 treated

411 with SB431542. **C.** The metastatic potential as reflected by matrigel traversal of A549
412 decreased following CBP-induced inhibition of CREB and TGFR blockade by
413 SB431542. * $p < 0.05$ compared to HT-29 and A549 and ** $p < 0.005$ compared to
414 HT-29 and A549.

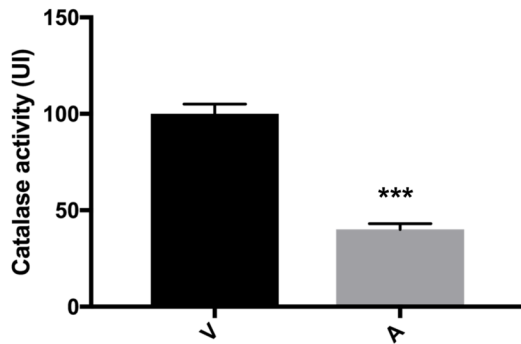
415 **Figure S1:**

416 **TGF- β 2 regulates oxidative stress in *Theileria*-transformed macrophages. A and**
417 **B.** Catalase activity is augmented in independent clones (H7 & H8) of macrophages
418 isolated from disease-susceptible Holstein-Friesian (H) animals compared to
419 independent clones (S2 & S3) of macrophages isolated from disease-resistant Sahiwal
420 (S) animals. SB431542 blockade of TGF-R abolished heightened catalase expression
421 by independent clonal lines of H macrophages, whereas adding TGF- β 2 to
422 independent clonal lines of S macrophages increased catalase activity. **C.** H₂O₂ output
423 is higher in disease-resistant S macrophages compared to disease-susceptible H
424 macrophages. Blockade of TGF-R-signalling in H macrophages increased levels
425 H₂O₂, while stimulating independent clonal lines of S macrophages with TGF- β 2
426 decreased H₂O₂ levels. *** $p < 0.001$ compared to Holstein macrophages and ###
427 $p < 0.001$ compared to Sahiwal macrophages.

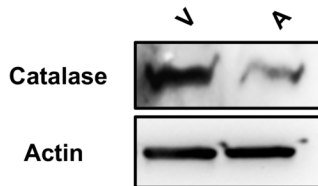
A

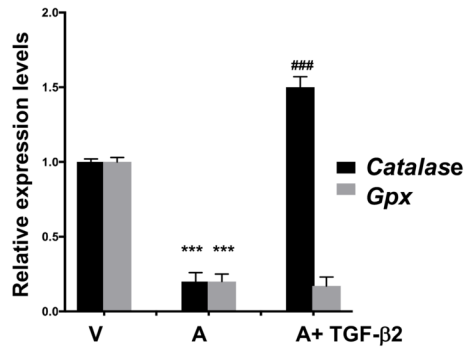
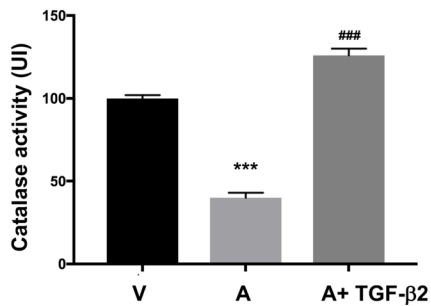
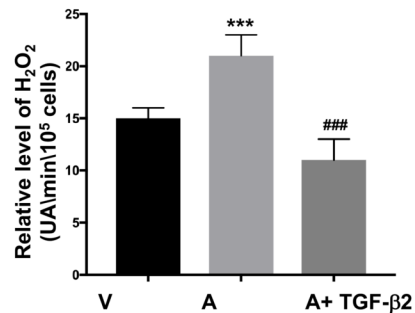


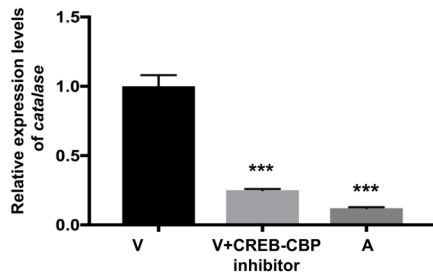
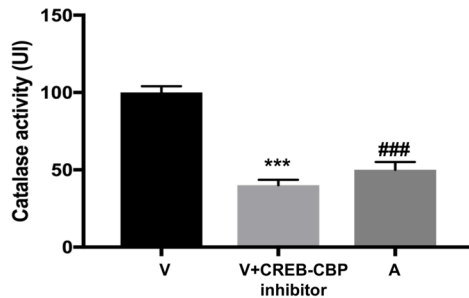
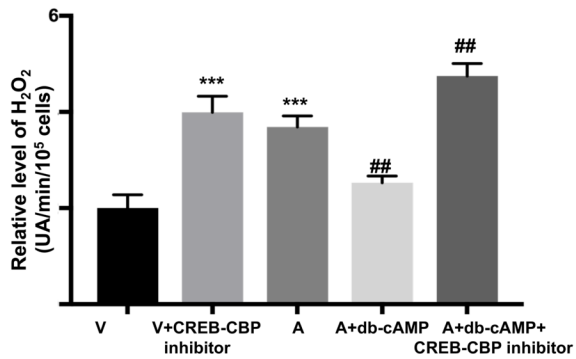
B

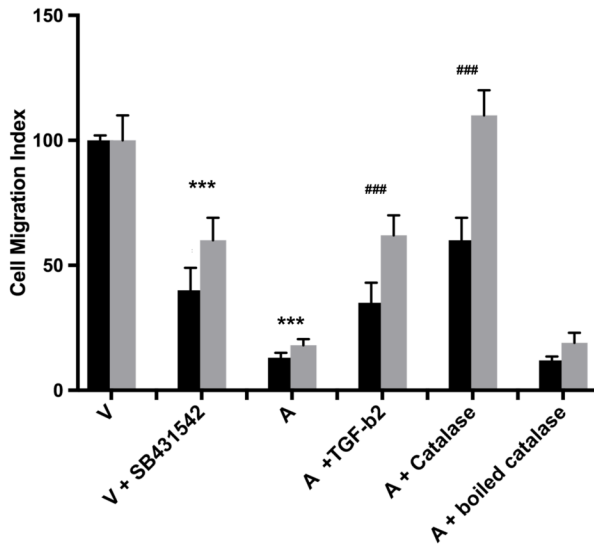
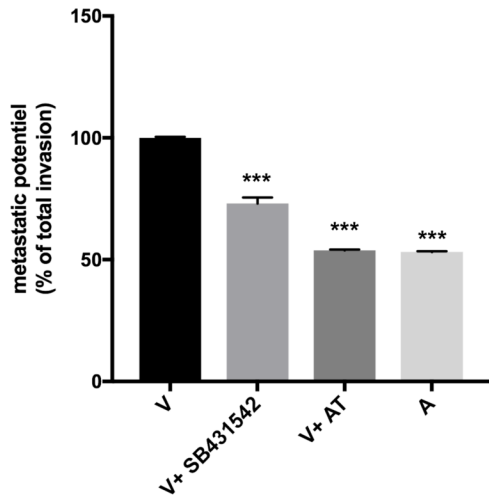


C

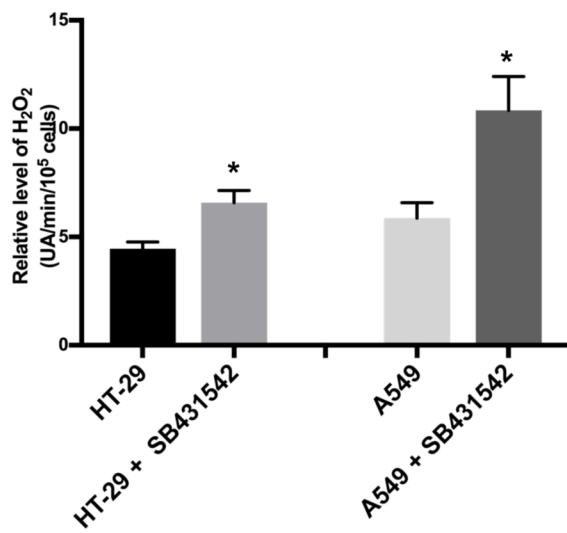


A**B****C**

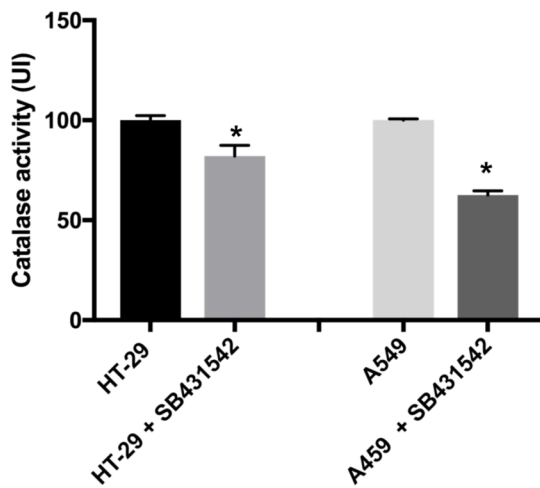
A**B****C**

A**B**

A



B



C

